

---

# **WATER QUALITY PROGRAMS DIVISION**

Standard Operating Procedure for the Collection of  
Macroinvertebrates in Streams

Revised and Adopted May 2013

*Draft Copy*



**OKLAHOMA WATER RESOURCES BOARD  
WATER QUALITY PROGRAMS DIVISION  
3800 NORTH CLASSEN  
OKLAHOMA CITY, OK. 73118**

**STANDARD OPERATING PROCEDURE FOR THE COLLECTION OF  
MACROINVERTEBRATES IN STREAMS<sup>1</sup>  
REVISED AND ADOPTED MAY 2013  
(PREVIOUS REVISION APRIL 2010)**

**1.0 General Information**

Most free flowing water bodies, with acceptable water quality and habitat conditions, support diverse macroinvertebrate communities in which there are a reasonably balanced distribution of species among the total number of individuals present. Macroinvertebrate community responses to environmental perturbations are useful in assessing water quality and habitat impacts. The composition and density of macroinvertebrate communities in flowing water are reasonably stable from year to year. However, seasonal fluctuation associated with life-cycle dynamics of individual species may result in extreme variation at specific sites within any calendar year. Assessing the impact of pollution generally involves comparison of macroinvertebrate communities and their habitats at sites influenced by pollution with those collected from adjacent unaffected sites.

Macroinvertebrate collections, for purposes of stream assessment, are made from the community that requires or prefers flowing (lotic) water. Reasons why this community type is sampled rather than various lentic communities include:

1. The flowing water community is routinely exposed to the average water quality of the stream;
2. The metrics used to analyze the macroinvertebrate community of streams were designed for the flowing water community;
3. The database of pollution tolerance of macroinvertebrates found in Oklahoma is much larger for lotic communities; and
4. The organisms most sensitive to water quality degradation tend to live in flowing water.

Due to these factors, the flowing water community is more suitable for assessing the condition of a stream, than by looking at the pool community where more tolerant organisms are found regardless of the stream's water quality. Lotic communities require a substrate of some type to attach to. The most common substrates of this type include rocky riffles, streamside root masses, and woody debris. Where possible, a rocky riffle should be sampled, but if it is not present, or is of dubious quality, if rocky riffles cannot be found at all streams of a given ecoregion, both of the other two alternate habitats should be sampled. The sampling methodologies for these habitat types are included in this SOP.

---

<sup>1</sup> Much of this SOP is adopted from four documents: Oklahoma Water Resources Board (1999) Technical Report 99-3: Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development, Oklahoma City, OK; Oklahoma Conservation Commission (2001), Standard Operating Procedure for Macroinvertebrate Collection, Subsampling, and Picking. Oklahoma City, OK.; United States Environmental Protection Agency (2008 and 2013). National Rivers and Streams Assessment Field Operations Manual, Washington, DC.

Invertebrate communities are constantly changing throughout the year as species emerge and new species hatch. Consequently, it is not possible to infer water quality from the invertebrate community of a stream by comparing it to a reference stream community that was collected at a different time of year. The springtime communities are especially unstable as many of the insects that over-winter as larvae begin to emerge. By summertime however, the insects that only have one generation per year have mostly emerged and the insects left are ones that hatch repeatedly throughout the summer. This means that collections made during different times of the summer tend to be the same in lieu of water quality differences. This period of the summer when collections from different streams can be compared to each other is termed the Summer Index Period.

OWRB's macroinvertebrate collection methodologies use modifications of several well-cited and published protocols including, OWRB Technical Report 99-3 (OWRB, 1999), the EPA Rapid Bioassessment Protocol V (USEPA, 1989) and the National Rivers and Streams Assessment (NRSA) Field Operations Manuals (USEPA, 2008 and 2013). Generally, OWRB protocols for wadeable waterbodies mirror the semi-qualitative RBP protocols, in addition to quantitative riffle kicks. For some studies, OWRB personnel may use the transect based methods described in the NRSA Field Manuals. For large wadeable and non-wadeable rivers, OWRB protocols conceptually mirror what has been developed for national studies, such as the NRSA, and the OWRB staff has been integral in developing these national protocols. Therefore, much of what is used at a state level is reflected nationally. Although Oklahoma's ecological diversity is unique, national protocols are developed to reflect a much broader range of conditions than what can be found in Oklahoma. So some notable differences do exist that can affect effort, and include: 1) targeting habitats as well as transect substrates, 2) compositing collections in coarse substrate rivers, and 3) moving outside the immediate transect and into the substrate to find representative habitats.

## **2.0 Definitions/Terms**

- Team Leader—crew member of fish collection team who provides support, expertise, and opinions; gives instruction and has final say on how work will be done; must score a 95% on critical fish identification
- Team Member—crew member of fish collection team who provides support, expertise, and opinions; follows the instructions of the team leader
- Riffle—any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. A riffle substrate must be composed of gravel, or cobble from 1" to 12" in the longest dimension; substrates of bedrock or tight clay are not considered suitable.
- Streamside Vegetation—any streamside vegetation that offers fine structure for invertebrates to dwell within or upon that receives suitable flow. Most habitats are located along undercut banks where fine roots of riparian vegetation are hanging in the water.

- Woody Debris—any dead wood with or without bark located in the stream with suitable current flowing over it.
- Summer Index Period—July 1 to September 15.
- Ethanol—preservative used in macroinvertebrate collections. Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant.

### **3.0 Safety**

Upon reaching the sampling location, site safety determinations should be made before proceeding. Please refer to the OWRB safety manual for instructions. General safety guidelines include:

- Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant.
  - Protective gloves and eyewear should be worn
  - Avoid inhalation of vapors

### **4.0 Quality of the Measurement**

#### **4.1 Training**

Principle investigators for the OWRB are required to have degrees and/or experience with biological or other applicable sciences. Principle investigators are defined as crew leaders, and this designation may be made upon the leader of a multi- or a one person crew. Training is required for all SOPs dealing with water quality and quantity collections and measurements as well as habitat assessments and biological collections. In-house training will be conducted for the use of all meters and digital titrators used for water quality or quantity measurements. Investigators must be familiar with OWRB SOP document and all training will follow the methods outlined in that document. Extra training will be provided when new SOPs are developed. Training of field crews will be done through dry run exercises in the laboratory to familiarize field crews with sample collection, sample preservation, instrument operation, calibration, and maintenance. In addition, when new personnel are hired or new methods developed, qualified staff will train on sample collection, measurement, and field analysis methods through side-by-side field trips. These trips will familiarize staff with SOP requirements. When training is considered adequate, a qualified staff member will check field staff for adherence to SOPs. Prior to collecting macroinvertebrates, subsampling, and picking, all staff should familiarize themselves with this SOP and OWRB Technical Report 99-3 Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development.

#### **4.2 Kinds of Quality Assurance Samples**

##### **4.2.1 Replicate Collections**

Replicate collection samples and duplicate subsampling and pick samples will be collected during each biological season. The scope and number of replicates will be determined by

the project Quality Assurance Project Plan. They may include replicates for various habitat or stream order.

#### **4.2.2 Vouchers and Photo-documentation**

All unique reaches and special or unusual circumstances should be photo-documented.

#### **4.2.3 Certification of Personnel for Laboratory Subsampling**

Benthic Macro-Invertebrate (BMI) samples are collected in the field, in accordance with Standard Operating Procedures. BMI samples returned to the laboratory consist of large amounts of detritus, algae, and other organic matter. Processing BMI data requires sorting through this Organic matter, in order to remove BMI for taxonomic identification. Many BMI are not easily recognized in and amongst the organic matter present in field-collected samples. In order to minimize variance in data created when sorting, Quality Assurance/ Quality Control measures will be implemented.

Quality Control of laboratory sorting will consist of a certification process for anyone who will sort field collected BMI samples. If Field samples are sorted by anyone not certified, a certified person must search the sorted organic matter for BMI before the sample is logged. No person, not certified, will discard any organic matter coming from a field sample that has not been inspected by a certified individual.

To minimize variability in BMI metrics there is a standard of quality, which will be required for certification. In order to be certified an individual must sort 4 out of 5 samples without overlooking more than 10 percent of all BMI present in the sorted portion of a field collected sample. The individual seeking certification should not discard any organic matter he/she has sorted. This organic matter will be saved and reviewed by a certified individual.

All organic matter sorted by an individual, seeking certification, will be retained and labeled with appropriate site information. The certifier will then examine the remaining organic matter for any invertebrates. A check will be run to determine if the sample will receive a passing mark. A minimum of 90% of the total number of individuals should be found by the individual seeking certification, in order to receive a passing mark. Additionally, one annual random QA check will be performed in each of the two years following certification. The same checks will be made to determine a passing mark. An index card (5" x 7") will be filled out to track the progress of each person seeking certification. Table 4.1 details the information required on the index card.

**Table 4.1: A template for Index card to Track progress of employee seeking certification for sub-sampling macroinvertebrates.**

Picker's Name: _____		Site Name: _____					
Section: _____		Project Code: _____					
Date	Habitat type	# individuals	Certifier's Initials	# Individuals	% missed	Taxonomic groups missed	Pass/Fail
Annual QA check: Pass or Fail		Certifier's Initials: _____					
Annual QA check: Pass or Fail		Certifier's Initials: _____					

**5.0 Personnel and Equipment**

**5.1 Personnel**

Macroinvertebrate collection crews may consist of one to two people. The team will consist of a team leader and possibly one team members. The team leader is someone with one or more seasons of collection experience. Collection experience in other programs may be substituted for that with the OWRB. The team leader will have the final say on all crew activities. A team member is someone trained on macroinvertebrate collection, subsampling and picking protocols. Team members will be expected to participate in the decision-making and follow the team leader’s direction.

**5.2 Equipment and Supplies**

**5.2.1 Kick Net**

OWRB uses a 1 m<sup>2</sup> kick net with 500 micron mesh. The kick net is composed of the net, brailles inserted and tied to the net, and a bottom leadline. The team leader will provide a detailed explanation of how to use the net. The kick net is used in riffles and only in flowing water. Bugs are collected by disturbing a 1 m<sup>2</sup> area upstream of the net by overturning, scrubbing, and agitating all material within the area. The small mesh selects for all bugs within the habitat.

The net should be checked regularly for holes and should be stored dry and free of snags and debris.

**5.2.2 Dip Net**

OWRB uses a modified dip net (a.k.a. D-net) with 500 micron mesh. The net is composed of a net attached to a D-ring and a one to three foot handle. The net is shrouded to protect against snags and increase longevity. The team leader will provide a detailed explanation of how to use the net. The dip net is used in streamside vegetation and woody debris and is only used in flowing water. Bugs are collected by disturbing the underside of root wads, undercut banks, wood, etc. The small mesh selects for all bugs within the habitat.

The net should be checked regularly for holes and should be stored dry and free of snags and debris.

### 5.2.3 General Supplies

#### Chemicals

Ethanol

#### Nets

Fishing line or dental floss to repair nets

Spare net

#### Containers

Wide mouth 1-quart polyethylene bottles and lids

Large bucket for in-stream subsampling

## 6.0 Collection of Macroinvertebrates

Before beginning collection, determine if flow conditions are suitable for collection. Samples must be collected in flowing water no greater than 3 cm (~1 inch) above the seasonal base flow. After a high flow event, at least 5 – 7 days should lapse before a collection is made to allow the benthic organisms to return to the preferred substrate. Additionally and when available, an established periphyton line should be used as an indicator of a stable baseflow condition. Furthermore, collection should be delayed for two weeks after a stream has gone from no flow (interrupted or dry conditions) to base flow conditions. If a stream is perennially non-flowing, or pooled, and no other arrangements can be made to visit the stream during a flowing period, collect the appropriate sample and make the note “**site not flowing**” or “**site pooled**” in the field notes, log in form, interior and exterior bottle labels, pick sheet, taxonomic field sheet, and final taxonomic record. These data may not be used for reporting but should be available for analysis.

Because sampling protocols involve the collection of material over multiple substrates and areas, collections may be quite large. Samples should be thoroughly cleaned in the field so that a quart sample jar is filled no more than  $\frac{3}{4}$  full. Cleaning and removal of large or whole leaves, gravel, and sticks is necessary. However, in the event that more material must be preserved, use additional bottles to preserve the sample (labeling bottles 1 of 2, or 2 of 2, for example). In no instance should samples be divided and subsampled in the field. This is a laboratory activity and can be done, if necessary, by the sorting technician. The sample is then subsampled and picked in the laboratory.

OWRB's macroinvertebrate collection methodologies use modifications of several well-cited and published protocols including, OWRB Technical Report 99-3 (OWRB, 1999), the EPA Rapid Bioassessment Protocol V (USEPA, 1989) and the National Rivers and Streams Assessment (NRSA) Field Operations Manuals (USEPA, 2008 and 2013). Generally, OWRB protocols for wadeable waterbodies mirror the semi-qualitative RBP protocols, in addition to quantitative riffle kicks. For some studies, OWRB personnel may use the transect based methods described in the NRSA Field Manuals. For large wadeable and non-wadeable rivers, OWRB protocols conceptually mirror what has been developed for national studies, such as the NRSA, and the OWRB staff has been integral in developing these national protocols. Therefore, much of what is used at a state level is reflected nationally. Although Oklahoma's ecological diversity is unique, national protocols are developed to reflect a much broader range of conditions than what can be found in Oklahoma. So some notable differences do exist that can affect effort, and include: 1) targeting habitats as well as transect substrates, 2) compositing collections in coarse substrate rivers, and 3) moving outside the immediate transect and into the substrate to find representative habitats.

### **6.1 Designation of Reach Length (All Biological Sampling Activities)**

Before sampling begins, a waterbody should be classified based on size and accessibility. Average wetted width (AWW) and fish gear will be used to determine reach length and whether a site is wadeable or boatable. First, a total possible reach length will be established by using either detailed aerial photos or direct measurement at the site. A total of five representative widths are used to obtain the AWW and should represent the diversity of the site. It is important to measure areas of varying width including bends, large shallow runs, and riffles. Areas directly around a bridge should be avoided. After the AWW is calculated, the reach length is set at 40x wetted width and rounded to the nearest "10". Second, fish gear accessibility is determined. If a reach is continuously wetted and greater than 50% of contiguous length can be safely and efficiently fished using a seine or pram/backpack electrofishing equipment, the site is classified as wadeable. Conversely, if a reach is continuously wetted and greater than 50% of contiguous length requires at least a 14-foot electrofishing boat to be efficiently fished and can be safely and efficiently accessed, the site is classified as boatable. Based on this information, the following rules should be used to determine reach length.

1. All sites will have a minimum reach length of 150 meters, regardless of wadeability.
2. Wadeable sites can be fished efficiently and effectively with pram or seine over greater than 50% of contiguous wetted length. Maximum reach length will be 2000 meters, regardless of calculated reach length.
3. Boatable sites can be fished efficiently and effectively with a boat over greater than 50% of contiguous wetted length. Maximum reach length will be 4000 meters, regardless of calculated reach length.
4. The reach length minimum and maximums can be adjusted under extremely unique circumstances, but only after prior consultation with the Monitoring Coordinator and Biological Team Leader and approval.

For large waterbodies, accessibility is not only a safety concern but should be used to help define reasonable effort. Merely because a boat could move unfettered through 3-4 feet of depth, the site is not by default considered boatable. Likewise, merely because a pram could be used to fish the near shore areas of a large river, a particular site should not by default be classified as wadeable. Best professional judgment should be used to determine which method would be most efficient and representative of the site. Furthermore, for permanent ambient trend sites or regularly visited sites, a chart is included in Appendix A denoting the average length calculated and the preferred sampling protocols for BMI, fish, and habitat. These recommendations take into account certain “tweener” sites that are difficult to classify as well as maintain consistency with site-specific methodology used to collect past samples.

## 6.2 Selection of BMI Sampling Method

Once reach length and the wadeable/boatable determinations are made, BMI sampling protocol will be determined. The OWRB uses either a long-standing rapid bioassessment protocol or a large river collection protocol, and regardless of size or accessibility, a riffle sample will always be taken if available. In Oklahoma, wadeable sites have been sampled for BMI for more than two decades using a timed protocol that targets the best available habitat. Because this protocol has been used to determine reference condition as well as use assessments, BMI collections on many wadeable waterbodies will continue utilize the RBP sampling methodology. For the NRSA, the differentiation between small and large wadeables occurred at an AWW of 12.5 meters, or 500 meters total reach length. In Oklahoma, the cutoff for RBP protocols has historically been an 800 meter calculated reach (OWRB, 2009). However, arbitrary cutoffs potentially bias sampling effort and sites may overlap a cutoff point depending on season or sampling year. Therefore, the current protocol provides a cutoff range set between reach lengths of 800-1000 meters. When sites fall in this range, team leaders can use best professional judgment to determine which protocol to use. Also, to facilitate this process, Appendix A provides the historically used protocol for regularly visited sites and can be used as a guideline for new sites. Based upon the above guidelines, the following rules should be used to determine reach BMI sampling methodology.

1. **The Rapid Bioassessment Protocol** applies to all waterbodies with calculated reach lengths less than 800 meters.
2. **The Large River Protocol** applies to all waterbodies with calculated reach lengths greater than 1000 meters.
3. **Best Professional Judgment** should be used to determine the most appropriate protocol on sites with calculated reaches of 800-1000. Among other things, the relationship to previously sampled sites as well as the density and regularity of targeted habitats can be used to assist this judgment. Consideration should be given to whether a transect based or reach wide timed sample would create a more representative sample. On larger waterbodies, the timed RBP approach may not adequately represent the diversity of available targeted habitats.
4. **Appendix A** will be followed for all regularly visited sites.

5. **Riffle collections** will be made whenever available regardless of reach length or accessibility. This may require going outside of the sampled reach.

If possible, reaches should avoid the influence of road crossings and be established in away from bridge influences in an upstream direction. As a rule, reaches will be upstream unless intervening factors are present (e.g., safety concerns, obstructions to passage, or confluence with a major tributary). In the event that crews cannot set the reach wholly upstream, it is preferable that the reach be established in a wholly downstream direction. As a last resort, crews may straddle the bridge. Record reach length and sampling direction on the appropriate habitat form (Figures 2 and 3).

Appropriate protocols will be selected based upon reach classifications. The rapid bioassessment protocol is explained in section 6.4. The large wadeable protocol will use one of two variations based upon whether the substrate is predominately fine (section 6.5.1) or coarse (section 6.5.2). Protocols are variations of the USEPA's National Rivers Streams Assessment Large River Macroinvertebrate Protocol. Regardless of classification or dominant substrate, a riffle sample will be collected when available (section 6.3). When sampling is complete, ensure that the appropriate habitat forms are completed (Figures 2 and 3).

**NOTE: In certain instances, the protocols discussed in the following sections may not follow real-world scenarios. For example, material to sample may be particularly sparse. Under those circumstances, err conservatively and collect the sample that is available. Note on the form what issue was faced (e.g., "wood was extremely sparse") and discuss the collection with the biological team leader and group supervisor. A decision can then be made in-house on how to proceed.**

### **6.3 Collection from Rocky Riffles (all waterbody classes)**

#### **6.3.1 Suitable Substrate**

A riffle is defined as any sudden downward change in the level of the streambed such that the surface of the water becomes disrupted by small waves. For this collection method the substrate of the riffle must be composed of gravel, or cobble from 1" to 12" in the longest dimension. Riffles with substrates of bedrock or tight clay are not suitable. Sand and balled clay may be suitable substitutes.

#### **6.3.2 Where to Sample the Riffle**

Three 1  $m^2$  areas of the riffle must be sampled. They can be square, rectangular or trapezoidal so long as each area equals 1  $m^2$  in area. One should be in the fastest part of the riffle where the largest rocks and the smallest amount of interstitial sediment will generally be found. The second should be in the slowest part of the riffle, often near the edge of the stream where the smallest rocks and the greatest amount of interstitial sediment will be found. The third sample should be in an area intermediate between the first two.

### **6.3.3 Method of Collecting the Sample (all waterbody classes)**

Support a 1-m<sup>2</sup> kick net composed of a double layer of fiberglass window screen or a net of number 30 mesh in such a way that the current will carry any organisms dislodged from the substrate into it. The bottom of the net should be tight against the bottom of the stream and the current must be sufficient to insure that dense organisms such as small mollusks will be carried into the net from the sampling area. There is no definite cutoff for stream velocity in the sampling area, but if possible, riffles with average velocities of 1 foot/second or greater are preferred and should be chosen if possible.

By kicking the substrate, vigorously agitate the substrate of a 1 m<sup>2</sup> area of the bed of the riffle immediately upstream of the riffle until all rocks and sediment to a depth of at least five inches have been thoroughly scraped against each other. Organisms living between and upon the rocks will have been dislodged and carried into the net by the current. Any rocks too large to kick should be brushed by hand on all surfaces. This can be done using your hands or with the aid of a brush. If a brush is used, you must be very careful to clean it after each site to prevent contamination of the next sample with invertebrates from the previous site. Continue agitation and brushing until it can be seen that the area being sampled is producing no new detritus, organisms, or fine sediment.

At this point, rinse leaves, sticks and other large debris caught in the net in the current in a manner such that organisms on them are carried into the net. When the volume of the sample is reduced so that three 1 m<sup>2</sup> samples will loosely fill a 1 quart jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar (sample type = Riffle). In no case should the jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. If necessary, preserve sample in several jars (note: this is not preferable and should only be done after sample is thoroughly cleaned). Label the sample appropriately following the instructions presented in section 6.5 Sample Handling & Preservation. In the event that more than one bottle is necessary, each jar should be labeled the same with the exception of bottle number (e.g., bottle 1 of 2 or bottle 2 of 2). Complete habitat form in Figure 2. Use the notes section to comment on any deviation from protocol including sparseness of material, lack of light or heavy flowing areas, etc.

### **6.4 Rapid Bioassessment Protocol**

For some wadeable streams, the collection of macroinvertebrates involves collection in three possible lotic habitats—riffles, streamside vegetation, and woody debris. The combination of methods was selected in order to produce a representative macroinvertebrate collection. Because Oklahoma streams vary widely in substrate, riffle habitats may not always be available. However, if a riffle is available, it should always be sampled whether the stream is classified as wadeable or non-wadeable. Sequence for collection is determined by site characteristics and crew leader

preference. However, care should always be taken not to disturb one substrate while sampling another. In general, the riffle should always be identified and sampled first.

#### **6.4.1 Collection from Streamside Vegetation**

Any streamside vegetation in current that offers fine structure for invertebrates to dwell within or upon is suitable. The vegetation being sampled must be in the current so that it offers suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. This habitat will often be found along the undercut banks of runs and bends where the fine roots of grasses, sedges, and trees, such as willow and sycamore, hang in the water.

This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the vegetation being sampled. The organisms can be dislodged from the roots either by vigorously shaking the net around the roots or by shaking the roots by hand while the roots are inside the net.

Sampling should continue for **3 minutes** of active root shaking. Do not count the time that elapses between sampling areas. Be careful to only sample roots in current. Usually, only one or two sides of a given root mass are in current. Be careful not to sample the backside of a root mass that is in still water.

At this point, rinse leaves, sticks and other large debris caught in the net in the current in a manner such that organisms on them are carried into the net. When the volume of the sample is reduced so that three 1  $m^2$  samples will loosely fill a 1 quart jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar (sample type = RBP-SSV). In no case should the jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. If necessary, preserve sample in several jars (note: this is not preferable and should only be done after sample is thoroughly cleaned). Label the sample appropriately following the instructions presented in section 6.5 Sample Handling & Preservation. In the event that more than one bottle is necessary, each jar should be labeled the same with the exception of bottle number (e.g., bottle 1 of 2 or bottle 2 of 2). Complete habitat form in Figure 2. Use the notes section to comment on any deviation from protocol including sparseness of material, lack of fine roots, etc.

#### **6.4.2 Collection from Woody Debris**

Any dead wood with or without bark in the stream is suitable as long as it is in current fast enough to offer suitable habitat for organisms which collect drifting particles or which need flowing water for other reasons. The final sample should consist of organisms collected from an even mixture of wood of all sizes and in all stages of decay.

This type of sample should be collected with a dip net made of #30 size mesh material. The net should be placed around or immediately downstream of the debris being sampled. The organisms can be dislodged from the debris either by vigorously shaking the net around the woody debris or by shaking the debris by hand while the debris is inside the net. Large logs that are too big to shake should be brushed or rubbed vigorously by hand while the net is held immediately downstream.

Sample for total of **5 minutes** counting only the time that debris is actually being agitated. Include as many types of debris in the sample as possible. These types often include wood that is very rotten and spongy with or without bark, wood that is fairly solid which has loose and rotten bark, wood that is solid with firmly attached bark, and any combination of these states. They should range in size from 1/4" to about 8" in diameter.

At this point, rinse leaves, sticks and other large debris caught in the net in the current in a manner such that organisms on them are carried into the net. When the volume of the sample is reduced so that three 1 m<sup>2</sup> samples will loosely fill a 1 quart jar three fourths (3/4) full or less, remove all of the material from the net and place it in the jar (sample type = RBP-Wood). In no case should the jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. If necessary, preserve sample in several jars (note: this is not preferable and should only be done after sample is thoroughly cleaned). Label the sample appropriately following the instructions presented in section 6.5 Sample Handling & Preservation. In the event that more than one bottle is necessary, each jar should be labeled the same with the exception of bottle number (e.g., bottle 1 of 2 or bottle 2 of 2). Complete habitat form in Figure 2. Use the notes section to comment on any deviation from protocol including sparseness of material, little wood decay, etc.

## **6.5 Large River Protocol**

Unlike the RBP method which samples over the entire reach, the Large River Protocol is a transect based methodology that samples at 11 equidistant transects. To establish transect length, divide total reach length by 10 (e.g., reach length = 400m, therefore, transect length = 40 m). The protocol requires that a collection(s) be made at each of the 11 transects, and that each type of collection be composited into a single sample. The reach should be laid out as illustrated in Figure 1. The upstream most transect (A) will be sampled on a randomly chosen bank. All other banks will be sampled in the order as seen in Figure 1 proceeding downstream through transect K. Depending on the dominant substrate, the fine or coarse substrate variation of the large river protocol will be used. However, regardless of substrate, both large river protocols have common characteristics.

1. Both methods sample two distinct zones within the river—the dominant substrate (fine or coarse) and a targeted habitat (e.g., decaying wood, fine roots, or vegetation).
2. A 1 linear meter sweep (timed at 30 seconds) is used to sample each zone. Sampling depth should not exceed 1 meter.
3. A 10 m x 20 m sampling plot is established at each transect (Figure 1). The dominant substrate sweep will **always** occur within this plot.
4. The targeted habitat sweep should be made within the 10 x 20 plot. However, if targeted habitats are not present, it may occur at any place throughout the sub-reach down to the next transect. For transect K, use a sub-reach length area around the transect. The targeted habitat types for both variations of the protocol are decaying wood, fine roots, or vegetation (submerged, emerged, or overhanging). Sample wood only greater than 5cm in diameter and a decayed indentation of at least 0.5mm.
5. If bedrock is the dominant bottom substrate in a 10 x 20 plot, it should be sampled as the dominant bottom substrate if it is present across > 50% of the wetted width. If not, sample either the fine or coarse substrate along the other bank.
6. If the bank chosen according to Figure 1 is too deep to sample within the plot, move to the other bank and sample the dominant bottom substrate. If the other bank is too deep, begin working toward the next transect until a suitable plot is available.

Major differences in the two protocol variations are:

1. **Types of bottom substrates**—For the fine substrate protocol, dominant substrates will be muck, silt, sand, fine gravel (up to 16mm or lady bug size), or hardpan clay. Conversely, for the coarse substrate protocol, dominant substrates will be coarse gravel, cobble, and boulder substrates.
2. **Sample Composition**—For the coarse substrate method, all substrate and targeted habitat sweeps will be composited into one sample and preserved. Conversely, for the fine substrate protocol, the substrate and targeted habitat samples will be kept separate and preserved as two distinct samples.

### 6.5.1 Large River—Fine Substrate Protocol

Fine substrate is defined as muck, silt, sand, fine gravel, hardpan clay, or a combination of 2 or more. This protocol will be used when > 50% of the reach substrate can be classified as fines. The following steps should be taken to collect sample:

1. Beginning at transect A (upstream), a random bank should be chosen and a 10m X 20m plot established.
2. At a distance of 1 meter downstream of the transect, collect a 1-meter linear sweep using a D-frame net with 500 um mesh. Sampling depth should not exceed 1 meter. With the net mouth oriented upstream, kick the bottom substrate while sweeping through it multiple times over a 30 second period. To avoid collection of excess material, avoid using the net as a dredge. To avoid an over collection of unfilterable sandy material while sampling in faster flows, kick in an area further upstream of net so that much of the non-organic material will

fall out before entering net. After the collection is made, sweep through the water several times to filter as much material as possible. Also, sort out any large material such as leaves, gravel, or other debris. At various points during the reach, it may be necessary to deposit material in a sieve and further filter fine particles by gently agitating the bucket through the water column or by tapping the net under water.

3. Within the 10m x 20m plot, determine the dominant targeted habitat. If targeted habitats are not present within the plot, you may find other material throughout the sub-reach down to the next transect. For transect K, use a sub-reach length area around the transect. First, look to the opposite bank in an equivalent plot area and then proceed in a downstream manner toward the next transect. If no targeted habitat is available on either bank for the entire sub-reach, then mark no collection on the form.
4. To collect the targeted habitat sample, use a different D-frame net to collect a 1-meter linear sweep over a 30 second period. If the material is decayed wood, time can be used to pick through the decayed portions to dislodge critters. As with the substrate sample, sort out any large material and deposit in a separate sieve bucket if necessary. Sample wood only greater than 5cm in diameter and a decayed indentation of at least 0.5mm.
5. Repeat the steps through transect K working in sequence outlined in Figure 1. When all transects are completed, composite all material from the dominant substrate sweeps into one sample (type = LRF-Sub) and all material from the targeted habitat sweeps into another sample (type = LRF-THab). When fines produce more material than  $\frac{3}{4}$  of a quart jar and further infield sorting cannot be done without compromising the sample, material may be divided amongst as many jars as necessary and labeled appropriately.
6. Label and preserve according to procedures in section 6.5. In no case should the jar be filled more than  $\frac{3}{4}$  full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. In the event that more than one bottle is necessary, each jar should be labeled the same with the exception of bottle number (e.g., bottle 1 of 2 or bottle 2 of 2).
7. Complete habitat form in Figure 3. Use the notes section to comment on any deviation from protocol including not sampling in the 10x20 plot, moving due to bedrock, etc.

### **6.5.2 Large River—Coarse Substrate Protocol**

Coarse substrate is defined as coarse gravel, cobble, boulder or a combination of 2 or more. This protocol will be used when > 50% of the reach substrate can be classified as coarse. The following steps should be taken to collect sample:

1. Beginning at transect A (upstream), a random bank should be chosen and a 10m X 20m plot established.
2. At a distance of 1 meter downstream of the transect, collect a 1-meter linear kick using a D-frame net with 500 um mesh. Sampling depth should not exceed meter. With the net mouth oriented upstream, kick and dislodge by hand the

material on the coarse substrate for 30 seconds, periodically sweeping through area to collect all dislodged organisms as the net is worked over the entire 1 meter length. To avoid collection of excess material, avoid using the net as a dredge. After the collection is made, sweep through the water several times to filter as much material as possible. Also, sort out any large material such as leaves, gravel, or other debris. At various points during the reach, it may be necessary to deposit material in a sieve and further filter fine particles by gently agitating the bucket through the water column.

3. Within the 10m x 20m plot, determine the dominant targeted habitat. If targeted habitats are not present within the plot, you may find other material throughout the sub-reach down to the next transect. For transect K, use a sub-reach length area around the transect. First, look to the opposite bank in an equivalent plot area and then proceed in a downstream manner toward the next transect. If no targeted habitat is available on either bank for the entire sub-reach, then mark no collection on the form.
4. To collect the targeted habitat sample, use a D-frame net to collect a 1-meter linear sweep over a 30 second period. If the material is decayed wood, time can be used to pick through the decayed portions to dislodge critters. As with the substrate sample, sort out any large material and deposit in a sieve bucket if necessary. Sample wood only greater than 5cm in diameter and a decayed indentation of at least 0.5mm.
5. Repeat the steps through transect K working in sequence outlined in Figure 1. When all transects are completed, composite all material all sweeps into one sample (type = LRC-Comp). If sample produces more material than  $\frac{3}{4}$  of a quart jar and further infield sorting cannot be done without compromising the sample, material may be divided amongst as many jars as necessary and labeled appropriately. Label and preserve according to procedures in section 6.5.
6. Label and preserve according to procedures in section 6.5. In no case should the jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample. In the event that more than one bottle is necessary, each jar should be labeled the same with the exception of bottle number (e.g., bottle 1 of 2 or bottle 2 of 2).
7. Complete habitat form in Figure 3. Use the notes section to comment on any deviation from protocol including not sampling in the 10x20 plot, moving due to bedrock, etc.

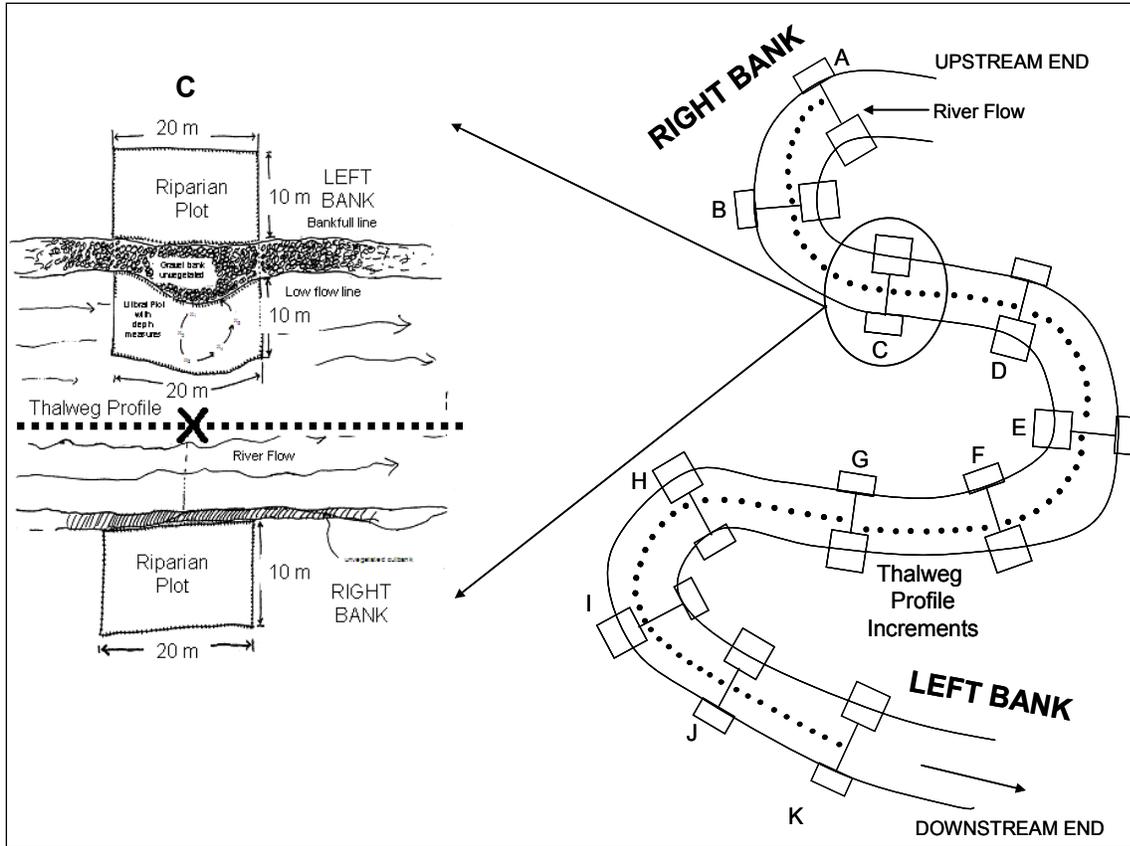


Figure 1. Illustration of sample reach layout and plot for Large River Protocol.

## 6.6 Sample Handling & Preservation

- **CAUTION:** Proper precautions should be taken when handling 100% ethanol. It is flammable, an intoxicant, and an eye irritant. Protective gloves and eyewear should be worn. Avoid inhalation of vapors.
  1. **Pack the Jar Properly.** In no case should the jar be filled more than 3/4 full of loose sample. Add 100% ethanol to the jar until the sample is covered and there is free ethanol on top of the sample. Gently invert and rotate the sample to ensure that ethanol completely inundates the sample, and add more ethanol if necessary. There should always be enough room in the jar to have at least 5 cm (2 inches) of free ethanol over the sample.
  2. **Label the Sample.** The jar should be labeled on the lid using a fine tip permanent ink marker (Sharpie) as described below. In addition, a small sheet of paper (approximately 2" x 2") should be filled out with the same information written in pencil and placed in the jar.

---

### Jar Lid & Sample Insert

---

Stream Name  
Sample Type  
Date of Collection  
Time of Collection  
Name of Collector(s)  
County  
Project Code

---

3. **Complete the Chain of Custody.** A Chain of Custody logbook is kept in the OWRB laboratory. Each jar should be entered into the COC as an individual sample. A sample number is assigned to each jar as it is registered on the COC. Sample numbers are added to labels and lids. Once samples are assigned a sample number and registered in the COC, they are stored in a refrigerator in the OWRB laboratory. Anytime a sample is removed from the refrigerator the COC will be updated to reflect this movement and track the sample.
4. **Transfer samples to the Macroinvertebrate Sample Custodian.** Correctly labeled macroinvertebrate samples, along with a COC form, should be transferred to the Project Manager for subsampling. The box should be conspicuously labeled with COC number. Once the samples have been received and the COC signed, the field sampler should make a photocopy of the COC for their records.

In some instances it may be necessary to drain the liquid from the sample and add fresh 100% ethanol. This is necessary when the sample contains a large amount of algae or other material with high water content or material that will rapidly become rancid. This will help preserve the morphological integrity of the invertebrates and greatly aid in taxonomic identification.

## **6.7 Subsampling and Picking of Macroinvertebrates from field Collected Samples**

The waterbody assessment procedure utilized by OWRB requires that a sample of macroinvertebrates be collected, identified and enumerated, from a 400-meter portion of the waterbody being assessed. In order to make this test cost effective it is not possible to identify more than about 150 organisms from each site. This procedure describes the process used to subsample a field-collected sample, which may contain 200-10,000 organisms.

1. **Remove a Field Sample from Storage.** The field collected macroinvertebrate samples will be transferred to the individual(s) designated to complete the macroinvertebrate sub-sampling. The individual who performs sub-sampling will initial and date the COC to indicate that a sample has been removed from the refrigerator. This individual is responsible for this sample until it is returned to the refrigerator and the COC is updated to annotate the samples return from sorting.

Samples will be sub-sampled in the order assigned on the COC form. If necessary to meet a reporting deadline or a data request, adjustments to this order may be made by a supervisor or project lead.

2. **Rinse Sample.** Without shaking or disturbing the contents, pour the liquid from the sample through a sieve made of #35 (500 micron) or finer screen. At this point, any silt, clay or fine sand in the sample should be GENTLY rinsed out of the sample. Be careful not to break off any of the delicate appendages that are used for identification of the animals. The sample will be easier to process if any large pieces of leaf, bark, stones, etc., are removed from the sample.
3. **Remove Large Pieces of Detritus and Sediment.** Large leaves and big pieces of wood and bark should be removed. Visually inspect all large detritus and pick all macroinvertebrates before removing detritus. At this point, the material remaining in the sieve should consist of a mixture of sand, fine gravel, small organic detritus, pieces of leaves < 1-2 cm wide, fine roots, algae and macroinvertebrates. Make sure to rinse away all silt and small sediments. Drain as much water as possible out of the sieve before transferring material to gridded tray for homogenization.
4. **Homogenize the remaining substrata.** Spread the sample out in a rectangular tray that is divided into 28 equally sized squares. A pan with a white background will help contrast between the organisms and the pan. A red permanent marker

is used with a straight edge to delineate square boundaries. Each square is assigned a number 1-28. Numbers do not need to be permanently marked on the pan. A diagram assigning each square a specific number will suffice. All detritus and sediment should be as uniformly distributed across the pan as possible. No water should be added to the sample at this point. Keeping the pan as dry as possible will prevent macroinvertebrates from drifting from square to square.

At this point the remaining sample material should be spread out across the sampling grid. The composition of substrata in each square should be equal to one another, as well as equal to the entire tray. For example if the whole sample is determined to contain 25% rotted leaves and 75% whole leaves, then 25% of each square should be rotted leaves and 75% should be whole leaves.

#### 5. Fill Out Sub-sampling/Picking Form.

- **Sample Number.** Write the sample number corresponding to the entry line in the Chain of Custody.
- **Site Name.** Write the name of the site as it is written on the sample jar.
- **Habitat Type.** Riffle, Streamside Vegetation, or Woody Debris.
- **Project Code.** Write the appropriate project code here.
- **County.** Write the Oklahoma County in which the sample was collected.
- **Legal Land Description.** Write the section number, township, and range associated with the stream reach where the sample was collected.
- **Collector.** Write the name of the person who collected the sample.
- **Collection Date.** Write the military date when the sample was collected.
- **Collection Time.** Write the 24-hour military time the sample was collected.
- **Name of Sorter.** Write the name of the person who performed the Sub-sample.
- **Date Sub-sampled.** Write the military date that sub-sampling occurred.
- **Estimate the Composition of the Sample.** Exclusive of invertebrates, estimate the composition of the sample according to the following list: silt and clay, sand, fine gravel (<2mm), coarse gravel (>2mm), woody debris (twigs, bark, roots, etc.), whole leaves, rotted pieces of leaves, filamentous algae, and unidentifiable organic material. Record the percentage of each fraction.
- **Square number.** Record the number of each square that was randomly selected for sorting.
- **List the Number of Animals Picked from each square.** Record the number of individuals removed from each square.
- **Simuliidae picked.** Record the number of black fly larva removed from each square.
- **Decapods picked.** Record the number of crayfish removed from each square
- **Large and Rare scan.** Record the number of individuals removed from the sample during the Large and Rare scan.

- 6. 100 count sub-sample.** Using the randomly generated numbers list provided in the macroinvertebrate data folder, select the next number from the list. Cross this number off. The contents of the selected square may be moved to a white sorting pan. In some instances organisms will be found with part of their body in one square and the rest in another. When this occurs move the organisms to the square in which, its head is found. Visually inspect the square in the gridded tray with a 10x lens to make sure no organisms were left in the selected square. Water may be added to the sorting pan to “float” organisms and assist in locating them. Locate and collect all the organisms in the sorting pan.

Keep track of the number of organisms picked. Place the organisms picked in a scintillation vial that is filled up to the neck with 80% ethanol and labeled “100 Count Sub-sample”. If any large organisms (too big to fit in the vial with the other organisms) are picked such as *Corydalidae* (hellgrammites), place them in a separate vial filled to the neck with 80% ethanol and labeled “100 count sub-sample (LG)”. If the number of organisms, placed in the scintillation vial does not exceed 100, then select the next number from the provided randomly generated numbers list. If the next number on the list corresponds to a square already removed from the current sample, then cross the number off and move to the next number on the list. Continue to remove the contents of each selected square, placing the contents in an empty white sorting pan. Remove all organisms from each selected square. When the number of organisms removed exceeds 100, continue to remove all organisms from the currently selected square. When all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, the 100-count sub-sample is complete. Label the “100 Count Sub-sample” vial appropriately and seal the vial. If any large organisms (too big to fit in the vial with the other organisms) are picked such as *Corydalidae* (hellgrammites), then label the vial appropriately and seal the vial.

**\*\*Note\*\*** *If a project specific QAPP or SOP calls for a larger fixed count sub-sample, then complete the first 100 count as described above. Make a new vial for subsequently removed organisms and continue as described in the 100 count protocol until all organisms have been removed from all randomly selected squares and the total number of organisms removed exceeds the target count. Seal the second vial and label it appropriately. If the project specific QAPP or SOP calls for a fixed count of 300 individuals label the vial 300ct. If the Project specific QAPP or SOP calls for a fixed count sub-sample of 500 organisms label the vial 500 ct.*

Special Considerations for 100 count sub-sample:

*Black fly (Simuliidae) larvae:*

Occasionally, field samples are dominated by Simuliidae (Black fly) larvae. It is argued that due to patchy distributions in nature, the entire stream reach may not truly be dominated by black flies. However, this patchiness may cause sub-samples to be inaccurately over populated by black fly larvae. This will in turn create a sub-sample not representative of the true population. Some protocols have been designed to account for this by sub-sampling 100 non black fly organisms (Oklahoma Conservation Commission 2001). It is also argued that exclusion of black flies from the 100 count will result in a sub-sample not representative of the true population. The Oklahoma Water Resources Board is currently investigating the effects that excluding black fly larvae will have on metrics used to describe macroinvertebrate community structure. If a project specific QAPP or SOP requires, then an extra step will be taken when performing the 100-count sub-sample. This extra step will allow metric to be calculated with and without black fly larvae.

If a project specific QAPP or SOP requires this extra step, black flies WILL be counted towards the 100 count. The number of black fly larva included in the 100 count will also be recorded. Once all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, the vial these organisms have been placed in is to be sealed and labeled "100 count sub-sample". At this point DO NOT move to the Large and Rare Scan.

Before moving to the large and rare scan, count the number of black fly larva included in the 100-count sub-sample. If the number of non black fly organisms removed, during the 100 count sub sample, exceeds 100, then there is no need for a further step. You may now move to the Large and Rare scan.

If the number of non black fly organisms removed, during the 100 count sub sample is less than one hundred, then continue sampling. Move to the next square, on the randomly generated numbers list, and remove all NON black fly larvae, counting them as you go. These organisms should be placed in a new vial filled to the neck with 80% ethanol and labeled "Non Black Fly Organisms". When all non black fly organisms have been removed from a square, add the number of non black fly larvae in both vials. If the number of non black fly organisms does not exceed 100, then continue to sub-sample all non black fly organisms by moving to the next square. Continue this process until the number of non black fly organisms exceeds 100. When the number of non black fly organisms exceeds 100, continue to remove all non black flies from the current square. Now label the "Non Black Fly Organism" vial appropriately and seal the vial.

*\*\*Note\*\* If project specific QAPP's and SOP's do not call for this extra step, then black flies WILL be counted towards the target count and added to the sub-sample vial like any other organism would be.*

*Decapoda (Crayfish)*

Dichotomous keys used to determine taxonomic designation for crayfish are written describing sexually mature males. To further complicate crayfish taxonomy, mature males change forms seasonally. Reliable keys used to identify 288 recognized species of crayfish, describe only breeding males (Form I). Additionally, 130 species have been described and recognized since the last revision of the most widely accepted key (Hobbs, 1972) The vast majority of crayfish collected are not Form I males and cannot reliably be identified.

Many small macroinvertebrates (e.g. Chironomidae) are often found attached to crayfish bodies. In order to avoid organisms attached to undersides of crayfish being overlooked, all crayfish are removed from selected squares and visually inspected for attached invertebrates. Crayfish ARE counted as part of the 100-count sub-sample. Invertebrates removed from crayfish bodies during this inspection are counted towards the 100-count sub-sample and tallied towards the square from which the crayfish was removed. The crayfish are placed into a separate vial filled to the neck with 80% ethanol and labeled with the appropriate sample number and the designation "Decapoda". Crayfish not found in selected squares are NOT removed from the gridded tray.

When all organisms have been removed from all randomly selected squares and the number of organisms removed exceeds 100, label the "Decapoda" vial appropriately and seal it. Crayfish are stored in a refrigerator in the OWRB laboratory. They are not sent to contracted taxonomists. Unless further reliable taxonomic resolution can be determined, crayfish are designated Decapoda for metric calculations.

- 7. 300 count sub-sample.** In order to gather more baseline macroinvertebrate data the OWRB may currently perform 300 count sub-samples on some or all collections. However, to keep these samples comparable to past samples and samples for other projects a 100 count sub-sample will be collected as well. Samples will be processed as if sampling to a 100 count sub-sample. Once the sorter has reached 100 organisms and no more organisms are present in the current square, no more squares will be added to the vial. That vial will be sealed and labeled as 100 count. At this point a new vial will be labeled 300 count. Any organisms collected after finishing the 100 count will be placed in this vial. Sub-sampling will not start over. The sorter will continue the sub-sampling procedure used for 100 count samples. The new target number of organisms becomes 300 or greater individuals (total between the two vials), however. The end product will be two vials, as well as a large and rare vial. The target end number for both vial should be 300. If in processing the 100 count sub-sample, greater than 300 individuals are picked due to density, that one vial will count for both the 100 and 300 sub-samples. Likewise, if in the process of picking the 100 count sub-sample the sample is "clean-picked", the one vial will count for both the 100 and 300 sub-samples .

8. **Large and Rare Scan.** Studies have shown that the number of organisms used in a fixed-count sub-sample has an effect on richness metrics used to describe community structure (Gowns et al. 1997). Countermach (1996) suggests that the chance of removing rare species, during fixed-count sub-sampling, increases as the number of total organisms removed increases. Debate over the optimal number of organisms to remove in a fixed-count sub-sample is ongoing in current literature and professional societies. Due to the increased cost of removing more organisms in a fixed-count sub-sample, many bio-assessment protocols use a lower number and a “Large and Rare Scan”. The purpose of this scan is to include large and rare species in sub-samples, without dramatically increasing resources needed to complete the sub-sample. The OWRB has adopted this practice and the protocol is described in this section.

The entirety of contents remaining in the gridded tray will now be scanned for large and rare organisms. Water may be added to the gridded tray to “float” organisms and assist in their location. Large organisms, for purposes of this scan, are defined as organisms large enough to be located without sorting through sample material. Rare organisms, for purposes of this scan, are defined as species not included in the 100-count sub-sample. The designation of “rare” species is more easily determined as experience sub-sampling increases. If you are not 100% certain if a species is rare, then consider rare.

Spend three minutes inspecting the remaining sample material for large and rare organisms. Place all organisms removed during the large and rare scan into a new scintillation vial, filled to the neck with 80% ethanol and labeled “Large and Rare Scan”. After three minutes have elapsed label the “Large and Rare Scan” vial appropriately and seal the vial.

9. **Label the Vial(s).** Each vial should be labeled on the lid, the side, and the interior. Use a fine point permanent ink marker to label each lid. Use a white sticky label on the side of each vial. Use an indelible ink pen and “write on rain” paper to create the interior label.

The lid of each vial should be labeled with the following information:

- Sample number
- “100 ct. sub-sample”, “100 ct. sub-sample (LG)”, “Non Black Fly Organisms”, “Decapoda”, or “Large and Rare Scan”
- Vial number (e.g. 1 of x, where x = total number vials for the sample number)

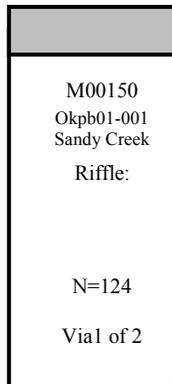
The side of each vial should be labeled with the following information:

- Sample number
- Project Code
- Water body name
- Habitat type (riffle, woody, vegetation)
- Number of individual organisms placed in the vial

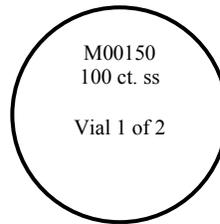
- Number of vials for this sample (e.g. 1 of x, where x = total number vials for the sample number)

The interior label should contain the following information:

- Sample number
- Project Code
- Water body name
- Habitat type (riffle, woody, vegetation)
- Name of Sub-sampler
- Date of Sub-sample
- Number of individual organisms placed in the vial
- Number of vials for this sample (e.g. 1 of x, where x = total number vials for the sample number)



Scintillation Vial



Scintillation Vial Cap

Figure Example labels for vial lids and exteriors.

**10. Place Clear Tape Over exterior Labels.** To protect the label from the ethanol, place clear tape (scotch tape) over the lid and side labels. This also ensures that the sub-sample has not been tampered with after it was completed. Vials, that do not provide vapor locking seals, should be sealed by tightly wrapping parafilm around the lid. This helps prevent evaporation of ethanol.

**11. Return Sub-sample vials to Storage.** Once macroinvertebrate samples have been picked and placed in properly labeled scintillation vials, the vials will be transferred to the laboratory refrigerator for storage. The individual who performed the sub-sample should update the COC, indicating the samples return to storage and the number of vials associated with the sample number. The responsibility for tracking of sub-sample vials is now returned to the Macroinvertebrate sample custodian.

**12. Remaining Sample.** The remainder of the field collected macroinvertebrate samples can now be discarded. No sample material will ever be discarded until an individual who is QA/QC certified for sub-sampling has investigated and signed off on the sub-sampling procedure.

## **7.0 Forms**

### **7.1 Field Notes**

Field habitat forms are documents used to annotate and record information that is gathered at the project site. They are a data sheet and should be treated as such. Therefore, they should be written, legible, and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. Field notes should be initialed and dated by the collecting personnel and data entry personnel. For guidance on proper procedure to complete the field notes, refer to your supervisor and or FTE. Field notes can be found at S:\Monitoring\STREAMS\biologica\biological planning\paperwork\. All field notes should be relinquished to the macroinvertebrate sample custodian, for appropriate filing and storage. The forms for this protocol are in Figures 2 and 3 at the end of this document.

### **7.2 Laboratory Log-in Sheets**

Log-in sheets are documents turned into the analytical laboratory for each sample collected. These forms are used to denote the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time of sample collection and be legible and complete. To avoid confusion and loss of data, a new sheet should be used at each new project site. For guidance on proper procedure to complete the log-in sheets, refer to your supervisor and or FTE. Log-in sheets can be found at S:\Monitoring\STREAMS\forms\.

### **7.3 Chains of Custody**

Chains of custody are documents turned into the analytical laboratory for each group of samples collected. These forms are used for several purposes. They act as a legal document to show proper delivery of samples occurred and they make a general list of the parameters that should be analyzed. They are a data sheet and should be treated as such. Therefore, they should include the date and time for each sample collected and is legible and complete. They should also be signed and dated by field and laboratory receiving personnel at the time of delivery. To avoid confusion and loss of data, a new chain of custody should be used for each group of samples. For guidance on proper procedure to complete the chains of custody, refer to your supervisor and or FTE. Chains of custody can be found at S:\Monitoring\STREAMS\forms\.

## **8.0 Data Storage**

All completed paper copies of forms and data sheets should be maintained with the appropriate station notebook. The data from the field notes and laboratory data sheets should be either entered into or uploaded to the Water Quality Biological Database. Each sample should be maintained electronically in the database under a unique sample number.

## 9.0 References

Countermach, D.L. 1996. Commentary on the subsampling procedures used for rapid bioassessments. *Journal of the North American Benthological Society* 15:381-385.

Hobbs, Jr., H.H. 1972. *Biota of Freshwater Ecosystems: Identification Manual No. 9. Crayfishes (Astacidea) of North and Middle America*. For the Environmental Protection Agency, Project # 18050 ELD.

Growns, J.E., B.C. Chessman, J.E. Jackson, AND G. Ross. 1997. Rapid assessment of Australian rivers using macroinvertebrates: cost and efficiency of 6 methods of sample processing. *Journal of the North American Benthological Society* 16: 682-693.

Oklahoma Water Resources Board (1999) Technical Report 99-3: Standard Operating Procedures for Stream Assessments and Biological Collections Related to Biological Criteria and Development, Oklahoma City, OK.

Oklahoma Conservation Commission, Water Quality Division, (2001) Standard Operating Procedures for Macroinvertebrate Collection, Subsampling, and Picking, Oklahoma City, OK.

United States Environmental Protection Agency. 1999. Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers, 2<sup>nd</sup> Edition, EPA 841-B-99-002, Office of Water, Washington, D.C.

United States Environmental Protection Agency. 2008. National Rivers and Streams Assessment Field Operations Manual, EPA 841-B-07-009, Office of Water, Washington, D.C.

United States Environmental Protection Agency. 2013. National Rivers and Streams Assessment Field Operations Manual, EPA 841-B-07-009, Office of Water, Washington, D.C.

# MACROINVERTEBRATE HABITAT ASSESSMENT

SITE NAME: \_\_\_\_\_ Site #: \_\_\_\_\_ Ave WW \_\_\_\_\_ Reach Length \_\_\_\_\_  
Upstream Lat/Long: \_\_\_\_\_ Downstream Lat/Long: \_\_\_\_\_  
COC (BUGS): \_\_\_\_\_ INVESTIGATORS: \_\_\_\_\_  
SITE DATE & TIME: \_\_\_\_\_ COLLECTION DATE & TIME: \_\_\_\_\_

## RIFFL

% OF SAMPLED COLLECTED: \_\_\_\_\_

UNIT OF EFFORT (M2): \_\_\_\_\_

### CPOM IN SAMPLE:

1. ABSENT (0%)
  2. SPARSE (>0, <5%)
  3. MODERATE (>5, <25%)
  4. ABUNDANT (>25%)
- EMBEDDEDNESS: \_\_\_\_\_ %

### SUBSTRATE TYPE & PERCENT

1. SILT & CLAY \_\_\_\_\_ %
2. SAND \_\_\_\_\_ %
3. GRAVEL \_\_\_\_\_ %
4. COBBLE \_\_\_\_\_ %
5. BOULDER \_\_\_\_\_ %
6. BEDROCK \_\_\_\_\_ %
7. HARD PAN CLAY \_\_\_\_\_ %

### SUBSTRATE ROUGHNESS

- 1 - LOW (>75% REMOVED)
- 2 - MODERATE (>25, <75%)
- 3 - HIGH (<25%)

### VELOCITY TYPICAL MAXIMUM

- 1 - LOW (0.2-0.5 FPS)
- 2 - MODERATE (0.5-1 FPS)
- 3 - HIGH (> 1 FPS)

### PERIPHYTON NON-CLADOPHORA

- 1 - SPARSE (THIN FILM)
- 2 - MODERATE (SLIGHTLY FUZZY <5MM)
- 3 - ABUNDANT (DEFINITELY FUZZY >5MM)

### CLADOPHORA

- 1 - ABSENT (0%)
- 2 - SPARSE (>0, <5%)
- 3 - MODERATE (>5, <25%)
- 4 - ABUNDANT (> 25%)

### AQUATIC MOSS

- 1 - ABSENT (0%)
- 2 - SPARSE (>0, <5%)
- 3 - MODERATE (>5, <25%)
- 4 - ABUNDANT (> 25%)

## STREAMSIDE VEGETATION

% OF SAMPLED COLLECTED: \_\_\_\_\_

UNIT OF EFFORT (MIN): \_\_\_\_\_

### CPOM (NON-ROOTS) IN SAMPLE:

1. ABSENT (0%)
2. SPARSE (>0, <5%)
3. MODERATE (>5, <25%)
4. ABUNDANT (>25%)

### PRESENCE

1. OCCASIONAL (>50 M FOR SAMPLE)
2. COMMON (10-50 M FOR SAMPLE)
3. ABUNDANT (10 M FOR SAMPLE)

### TYPE (> 1/4 OF SAMPLE)

1. GRASS-LIKE LEAVES
2. FINE ROOTS
3. COARSE ROOTS
4. LUDWIGIA STEMS (WATER WILLOW)

### VELOCITY TYPICAL MAXIMUM

1. LOW (0.2-0.5 FPS; 0.061-0.15 MPS)
2. MEDIUM (0.5-1 FPS; 0.152-0.305 MPS)
3. HIGH (> 1 FPS; 0.305 MPS)

### PERIPHYTON NON-CLADOPHORA

1. SPARSE (THIN FILM)
2. MODERATE (SLIGHTLY FUZZY < 5MM)
3. ABUNDANT (DEFINITELY FUZZY >5MM)

### CLADOPHORA

- 1 - ABSENT (0%)
- 2 - SPARSE (>0, <5%)
- 3 - MODERATE (>5, <25%)
- 4 - ABUNDANT (> 25%)

## WOODY DEBRIS

% OF SAMPLED COLLECTED: \_\_\_\_\_

UNIT OF EFFORT (MIN): \_\_\_\_\_

### CPOM (NON WOODY) IN SAMPLE:

1. ABSENT (0%)
2. SPARSE (>0, <5%)
3. MODERATE (>5, <25%)

### PRESENCE

1. OCCASIONAL (>50 M FOR SAMPLE)
2. COMMON (10-50 M FOR SAMPLE)
3. ABUNDANT (10 M FOR SAMPLE)

### SIZE

1. SMALL (0.6-2.0 CM)
2. MEDIUM (2.0-7.5 CM)
3. LARGE (>7.5 CM)

### STATE OF DECAY

1. LOW (INDENTATION 0-0.5 MM)
2. MEDIUM (INDENTATION 0.5-2MM)
3. HIGH (INDENTATION >2MM)

### VELOCITY TYPICAL MAXIMUM

1. LOW (0.2-0.5 FPS; 0.061-0.15 MPS)
2. MEDIUM (0.5-1 FPS; 0.152-0.305 MPS)
3. HIGH (>1 FPS; 0.305 MS)

### PERIPHYTON NON-CLADOPHORA

1. SPARSE (THIN FILM)
2. MODERATE (SLIGHTLY FUZZY < 5MM)
3. ABUNDANT (DEFINITELY FUZZY >5MM)

### CLADOPHORA

- 1 - ABSENT (0%)
- 2 - SPARSE (>0, <5%)
- 3 - MODERATE (>5, <25%)
- 4 - ABUNDANT (> 25%)

NOTES:

## MACROINVERTEBRATE HABITAT ASSESSMENT Large River Protocol Form

SITE NAME: \_\_\_\_\_ Site #: \_\_\_\_\_

Ave WW \_\_\_\_\_ Reach Length \_\_\_\_\_ Boatable YES/NO Dominant Bottom Substrate FINES (2 Samples)/COARSE (1 Sample)

Upstream Lat/Long: \_\_\_\_\_ Downstream Lat/Long: \_\_\_\_\_

COC (BUGS): \_\_\_\_\_ Samples Collected LRF-Sub/LRF-THab/LRC-Comp INVESTIGATORS: \_\_\_\_\_

SITE DATE & TIME: \_\_\_\_\_ COLLECTION DATE & TIME: \_\_\_\_\_

Transect	A	B	C	D	E	F	G	H	I	J	K
Location (L/R)											
Dominant Bottom Substrate											
Bottom Substrate Class											
Dominant Target Habitat											
Channel Code											
<b>Dominant Bottom Substrate</b> C = Coarse F = Fines L = Leaf Pack Matter B = Bedrock O = Other (make note)				<b>Dominant Target Habitat</b> WDm = Wood (moderate decay 0.5-2mm) WDh = Wood (high decay > 2mm) ME = Emergent Macrophytes MS = Submerged Macrophytes VO = Overhanging Vegetation FR = Fine Roots O = Other				<b>Channel Unit Code</b> P = Pool GL = Glide RI = Riffle RA = Rapid O = Other			
<b>Bottom Substrate Class</b> CL = Clay SI = Silt/Muck SA = Sand GF = Fine Gravel GC = Coarse Gravel C = Cobble BD = Boulder L = Leaf Pack B = Bedrock O = Other (make note)											

**Notes:** (PLEASE NOTE ANY TRANSECTS WHERE SAMPLE NOT TAKEN FROM INITIAL 10m x 20 m PLOT)

Appendix A.